REVIEW ARTICLE

Effects of Streptococcal Pyrogenic Exotoxin B on Pathogenesis of *Streptococcus pyogenes*

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Streptococcus pyogenes (group A streptococcus, GAS) is a ubiquitous and important human bacterial pathogen. This organism possesses several virulence factors to establish infection. One of these, the streptococcal pyrogenic exotoxin B (SpeB), is the predominant secreted cysteine protease of GAS. SpeB cleaves or degrades host serum proteins such as human extracellular matrix, immunoglobulins, complement components, and even GAS surface and secreted proteins. Destruction of both host and bacterial proteins makes SpeB the key virulence factor in GAS pathogenesis. Although several lines of evidence have shown that SpeB is an important virulence factor of GAS, its role in streptococcal infection remains controversial. Here, we review several publications and describe our current understanding of SpeB in GAS pathogenesis. [*J Formos Med Assoc* 2008;107(9):677–685]

Key Words: group A streptococcus, pathogenesis, SpeB

Streptococcus pyogenes (group A streptococcus, GAS) is an important Gram-positive human pathogen, which causes mild to severe diseases, including tonsillitis, pharyngitis, cellulitis, scarlet fever, rheumatic fever, necrotizing fasciitis, and streptococcal toxic shock syndrome (STSS).[1,2] In Taiwan, the most common clinical manifestation of GAS infection is skin and soft tissue infection.[3] The invasive GAS infections, such as bacteremia and STSS, are associated with a high mortality rate (25% for bacteremia and 66.7% for STSS).[3] In addition, GAS is one of the major pathogens that causes childhood bacterial pharyngitis in Taiwan.[4]

Many virulence factors of GAS have been identified, including bacterial surface proteins, hyaluronic acid capsule, secreted streptolysins, hyaluronidase, streptokinase, DNase, and many streptococcal pyrogenic exotoxins.[5] Streptococcal pyrogenic exotoxin B (SpeB) is one of the most important virulence factors of GAS. SpeB is the predominant extracellular protein in streptococcal culture supernatant. It is secreted as a 42-kDa zymogen and autocatalyzed into an active 28-kDa cysteine protease.[6,7] The conversion of the zymogen form of SpeB is a stepwise process that involves at least eight intermediates, with a combination of *cis*- and *trans*-processing.[6] Chen et al have speculated that the 28-kDa active form of SpeB is important for converting the 42-kDa zymogen to 28-kDa SpeB under physiological conditions.[6]

SpeB is a super-active protease with endopeptidase activity and has substrate specificity similar to that of the papain family proteases.[8] SpeB cleaves or degrades host extracellular matrix, immunoglobulins, complement components, and even GAS surface adhesins (M protein and protein F1),

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Table. Host and bacterial proteins cleaved by SpeB

Before SpeB cleavage	After SpeB cleavage	Potential effects	References
Host proteins			
Interleukin-1β precursor	Active interleukin-1β	Induce inflammation	14
Pro-matrix metalloprotease (pro-MMP 2, 9)	Active MMP (2, 9)	Enhance tissue damage and bacterial invasion	21, 22
Fibronectin	Fragmented	Participate in bacterial colonization and invasive infection	15
Vitronectin	Degraded	Enhance tissue damage	15
Kininogen	Bradykinin	Increase vascular permeability; induce fever and pain	23
Immunoglobulin (IgA, IgM, IgD, IgE, IgG)	Cleavage into Fc and Fab fragments	Inhibit immunoglobulin-mediated opsonophagocytosis	12, 24, 25
C3b	Degraded	Escape phagocytosis	26
Plasminogen	Degraded	Reduce plasmin activity on GAS surface	27
Bacterial proteins			
Zymogen form of SpeB	Active SpeB	Degrade or cleavage of bacterial and host proteins	6–8, 28
M protein	Remove 24 amino acids from N-terminus; release from bacterial surface	Alter immunoglobulin binding properties; promote bacterial dissemination	10, 29, 30
Protein F1	Degraded	Reduce bacterial internalization	11, 17
EndoS	Degraded	Lost IgG glycan-hydrolyzing activity	9
SmeZ	Degraded	Abolish immune stimulatory activity	31
Fba	Degraded	Inhibit binding of FH and FHL-1	32
C5a peptidase	Release from bacterial surface	Degrade chemotactic complement factor C5a	10, 33, 34
Streptokinase	Degraded	Unknown	13, 19
Protein H	Release from bacterial surface	Promote bacterial dissemination	10
Sda1	Degraded	Decrease neutrophil extracellular trap clearance	35

C5a peptidase, and several secreted proteins (Table).[6–35] Destruction of host defense system proteins and cleavage of GAS surface proteins may help the bacterium to escape immune clearance, invade the deeper tissues, and disseminate from the primary infection site. Furthermore, SpeB is also considered to be the major antigen involved in the pathogenesis of acute post-streptococcal glomerulonephritis (APSGN).[36–40]

Natural Sequence Variations

The *speB* gene is located on the bacterial chromosomal DNA and is present in all analyzed GAS strains. The nucleotide sequence of the *speB* gene is highly conserved. It is believed that the *speB* gene sequence variation is due to accumulation of point mutations.[15] In addition, the sequence variation among GAS strains has no apparent association with different clinical symptoms, geographic locations, or time periods.[15] Although all GAS strains have the *speB* gene, about 25–40% of analyzed strains express little or no SpeB protein.[41,42] Interestingly, Kansal et al[43] and Shiseki et al[44] have shown that more STSS-derived strains have little or no SpeB expression compared with noninvasive strains. However, the molecular mechanism and the biological significance of the SpeB non-secretor strains are still unknown.

The SpeB protein can be divided into three major forms, mSpeB1, mSpeB2 and mSpeB3,

according to the primary amino acid sequence (mSpeB1, S208 and A317; mSpeB2, G308 and A317; mSpeB3, S308 and S317).[45] The protease activity of these three variants is similar, however, only mSpeB2 has the human integrin ($\alpha_v\beta_3$ and $\alpha_{IIb}\beta_3$) binding domain, the arginine–glycine–aspartic acid (RGD) domain. Although the role of the mSpeB2 RGD domain in pathogenesis is not established, Stockbauer et al have proposed that the RGD motif of SpeB may participate in: (1) bacterial adherence to the host cell surface; (2) inhibition of platelet aggregation; and (3) enhancing its degradation efficiency by concentrating the SpeB at local infection sites.[45] In summary, although sequence variations (*speB* gene and SpeB protein) are found among different strains, the significance of these variations is not well established.

Disease Severity

In clinical epidemiology surveillance, the association of SpeB expression with disease severity in GAS infection is still controversial. The M1 type, the most dominant serotype of GAS infection, is significantly associated with higher protease expression.[42] In addition, high-level protease production is associated with severe symptoms including STSS, soft tissue necrosis and mortality.[42,46] Although these studies have suggested that SpeB is an important risk factor in severe GAS infection, the opposite results have been shown by other studies. Shiseki et al have reported that STSS-derived strains express lower levels of cysteine protease than do scarlet-fever-derived strains.[44] Similarly, an inverse relationship between disease severity and SpeB expression has been found in M1 isolates. This suggests that the decreased SpeB expression may help bacteria to maintain an intact surface M1 protein structure, which aids in resisting immune clearance.[43] These controversial results suggest that the severity of GAS infection does not depend on a particular virulence factor like SpeB. The interaction between bacterial virulence and host factors may determine the final outcome of infection.

Bacterial Internalization

Although GAS is considered an extracellular pathogen, recent studies have shown that GAS can internalize into epithelial cells. Strains isolated from bacteremia patients have shown internalization activity equal to that of intracellular pathogens such as *Salmonella* spp. and *Listeria monocytogenes*.[47] In clinical studies, intracellular GAS has been detected inside the epithelial cells in biopsies from GAS-infected patients biopsies or tonsils from asymptomatic carriers.[48,49] Osterlund et al have reported that GAS in epithelial cells was detected in 36% of asymptomatic carriers and in 93% of GAS-infected tonsillitis patients.[48,49] In addition, GAS has also been found inside human macrophage and macrophage-like cells collected from acute invasive diseases or asymptomatic GAS carriers.[49,50] In cell culture models, after eradication of the extracellular bacteria by antibiotic treatment, GAS can survive inside epithelial cells for 4–7 days.[51] When the antibiotics are removed from the culture medium, viable GAS can externalize and establish an extracellular infection, which causes cell death and monolayer damage.[51,52] These studies have indicated that GAS can survive inside epithelial or macrophage-like cells for a period of time, and externalize to cause recurrent infections. Intracellular GAS also contributes to penicillin resistance.[53] Penicillin is the first choice for treatment of GAS infection. Although there is no evidence to show that the minimum inhibitory concentration of penicillin for GAS is changing, the failure rate of bacterial eradication in streptococcal pharyngotonsillitis is 2–38%.[51,54] Kaplan et al have demonstrated that intracellular GAS is efficiently eradicated by either erythromycin or azithromycin, but not by penicillin, cephalosporin or clindamycin.[55] These studies suggest that an intracellular niche provides a barrier for GAS to escape killing by penicillin.

However, the role of SpeB in bacterial internalization is controversial. Tsai et al have shown that, although the adhesion ability of NZ131 (M49) and its *speB* isogenic mutant is similar, the *speB* mutant exhibits a two- to threefold decrease

in internalization into A-549 human alveolar carcinoma epithelial cell line.[56] In addition, the internalization activity is increased when cultured cells are infected by an *speB* mutant supplemented with purified SpeB protein.[56] Chaussee et al have further demonstrated that SpeB affects the fibronectin-mediated internalization pathway.[11] The major fibronectin-binding protein of GAS, protein F1, is efficiently degraded by SpeB. The degradation of protein F1 reduces bacterial fibronectin binding and internalization activities.[17] However, in other studies, inactivation of the *speB* gene has increased internalization into HEp-2 and Chinese hamster ovary cell lines.[11,57] These conflicting results may be caused by the different GAS strains and cell lines used in different studies. In general, it is believed that SpeB alters the bacterial surface proteins to affect the internalization efficiency, but which proteins, and how these surface proteins are involved in internalization, may be strain dependent.

Apoptosis

In addition to being responsible for recurrent infection and antibiotic resistance, internalized GAS also causes epithelial cell apoptosis. About 20% of A-549 cells and 11–13% of HEp-2 cells undergo apoptosis after GAS infection.[58] GAS internalization is required for inducing epithelial cell apoptosis. Therefore, the virulence factors responsible for GAS internalization, such as protein F1 and SpeB, are considered to have a critical role in apoptosis.[58,59] Recently, SpeB protease activity has been shown to be required for inducing apoptosis of cultured cells.[60] Mitochondrial dysfunction and upregulation of calcium-binding protein genes have been observed in SpeB-induced apoptotic cells.[61] In addition, the activation of both intrinsic and extrinsic caspase pathways has been observed, which indicates that SpeB may cause mitochondrial dysfunction and trigger apoptosis via the receptor-binding pathway.[61,62] Tsai et al found that the 28-kDa SpeB protease mutant protein induces apoptosis effectively, unless cells are pretreated with active SpeB protein, which suggests that SpeB-induced apoptosis is mediated through a receptor-like mechanism.[62] Further studies have identified integrin $\alpha_v\beta_3$ as the cell receptor of SpeB.[60] Mutation in the integrin binding site of SpeB (the RGD motif) partially decreases its induction of apoptosis. In addition, binding of Fas to SpeB has also been demonstrated.[60] Fas-Fas ligand binding is an important pathway for inducing apoptosis. Tsai et al found that anti-Fas antibody inhibits apoptosis induced by SpeB or the RGD motif mutant SpeB, which suggests that SpeB also induces apoptosis through the Fas-mediated apoptotic signaling pathway.[60] SpeB protein is internalized into epithelial cells via lysosome- and clathrin-dependent pathways.[63] However, internalized SpeB is degraded within 60 minutes after its contact with cells, which suggests that internalized SpeB may have no role in caspase activation.[63]

Purified SpeB protein also induces human monocyte-like U937 cells and human polymorphonuclear (PMN) cells to undergo apoptosis.[64–66] The cysteine protease activity of SpeB is important for triggering apoptosis in these cells.[65] In addition, the interleukin-1β converting enzyme family protease and Fas-mediated apoptosis pathway might be associated with the SpeB-induced apoptosis pathway, however, the mechanism is not clear.[65,66] Inducing apoptosis of phagocytic cells may contribute to evading immune clearance.

Evasion of Immune Clearance

SpeB is known to be an important factor in resisting clearance by phagocytosis and survival in host blood. In a mouse model, the *speB* mutant was eliminated effectively from the peritoneum after infection, but the wild-type strain was not.[67] In addition, the purified SpeB protein reduces U937 cell phagocytic activity and human PMN cell metabolic activity by causing mitochondrial damage.[65,68] While it directly causes PMN cell damage, SpeB also cleaves antibody and complement components to escape antibody- and

complement-mediated phagocytosis. Collin et al showed that SpeB cleaves IgG into Fc and Fab fragments.[24] Eriksson and Norgren showed that SpeB cleaves antigen-bound (Fab region-bound) IgG efficiently, but not Fc region-bound IgG.[12] This study indicates that SpeB recognizes and cleaves antigen-bound IgG to avoid Fcγ-receptor-mediated phagocytosis, and leaves nonspecific IgG on the bacterial surface as a host-like mask. In addition, recent studies have shown that several complement components are digested by SpeB. Complement component C3 is the central component of the complement activation cascade. It is digested by C3 convertase to generate C3b. C3b binds to pathogen surfaces to facilitate phagocytosis, initiate the alternative complement pathway, and amplify the effects of the classical pathway. Kuo et al found that SpeB cleaves serum C3, which impairs C3 fragment opsonization on the surface of GAS.[16] Terao et al showed that either recombinant SpeB protein or wild-type GAS strains rapidly degrade C3b.[26] In addition, SpeB releases the C5a peptidase from the GAS surface, to inactivate the leukocyte chemotaxis activity of C5a.[10] Properdin, a positive regulator of complement activation, is also degraded by SpeB.[69] These studies indicate that SpeB degrades important complement components, which impairs the complement activation pathway and allows GAS to evade opsonophagocytosis.

Animal Models

Although several lines of evidence indicate that SpeB is an important virulence factor of GAS, the results from mouse infection models are in conflict. Mice infected by M1, M3 and M49 *speB* mutants, through air pouch or intraperitoneal challenge, showed less mortality compared with that of the wild-type strains.[70,71] Supplementation with recombinant SpeB protein during *speB* mutant infection in the air pouch infection model increased mouse mortality.[70] The *speB* mutant also showed a decrease in bacteremia and dissemination to organs during infection.[67,72] When mice were infected subcutaneously, the *speB* mutant caused significantly smaller abscesses than those with the wild-type strain.[73] Finally, SpeB-immunized mice were protected from lethal wild-type strain challenge.[70] However, conflicting results have been observed in other studies. Ashbaugh and Wessels showed that the virulence of two *speB* mutants derived from the same parental strain was different.[74] Only one *speB* mutant showed less virulence for mice after intraperitoneal infection.[74] When compared with the wild-type strain, this *speB* mutant lost SpeB expression and decreased expression of its hyaluronic acid capsule. The other *speB* mutant, which had equal capsule expression to the wild-type strain, showed no significant decrease in virulence for mice, after intraperitoneal or subcutaneous infection. Saouda et al showed that injection of SpeB protein alone into the skin air sac did not cause significant tissue damage.[75] Tissue necrosis was increased and accelerated by infection of mice by SpeB in combination with GAS (wild-type strain or *speB* mutant). Both studies have indicated that one or more bacterial components, other than SpeB, are critical factors that cause severe GAS infection in mouse models.

Phase-shift of SpeB Expression

Passage of GAS in either human blood or mice induces alteration of bacterial colony morphology and the pattern of expression of virulence genes. Kazmi et al showed that upregulation of SpeA expression and downregulation of SpeB expression were observed after GAS passage in a mouse infection chamber model.[76] Raeder et al demonstrated that large- and small-colony variants are present after passage in human blood or mice.[77] The large-colony variants are SpeB negative, but the small-colony variants are SpeB positive. Because SpeB degrades many bacterial proteins, downregulation of SpeB expression *in vivo* may help bacteria establish successful infection by retaining their surface and secreted proteins.[78] Therefore, SpeB negative strains are considered to be the invasive

strains. Engleberg et al found that the large-colony variants (SpeB-negative strains) have a spontaneous mutation of the *covR/S* gene.[79] CovR/S, the best-characterized two-component regulatory system of GAS, is an important global regulator in GAS.[80,81] The strains with the *covR* or *covS* gene mutations show an increase in SpeA expression and a decrease in SpeB expression.[82] In addition, complementation of *covR/S* expression with a plasmid in SpeB-negative strains can restore SpeB expression.[82] These experiments have demonstrated that the *covR* or *covS* gene mutations are associated with loss of SpeB expression. In addition, the bacteriophage-encoded DNase (*sda1*) is essential for the phase-shift of SpeB expression (from SpeB-positive to -negative). Sda1 protects GAS against neutrophil clearance by degrading the neutrophil extracellular traps.[35] Abolition of SpeB expression may prevent DNase degradation, thus increasing the neutrophil resistance of GAS. Therefore, it is hypothesized that the *sda1* gene provides evolutionary selection pressure for switching SpeB expression during *in vivo* infection.[35]

SpeB and APSGN

GAS also causes non-suppurative sequelae such as rheumatic fever and APSGN. Rheumatic fever is a complication of throat infection and is most common among children between 5 and 15 years old. The clinical symptoms include multiple joint migratory arthritis, carditis, and rheumatic heart disease.[1,2] APSGN is an immune-complex-mediated renal disease, which is strongly associated with GAS infection.[37] These diseases are caused by autoantibody-mediated immune-complex pathogenesis after GAS infection.[38] The SpeB protein (zymogen and mature form) and anti-SpeB antibody are considered to be involved in APSGN pathogenesis.[36,37,40] Although several GAS antigens, included streptokinase, glyceraldehyde-3-phosphate dehydrogenase, and SpeB (zymogen and mature form) are considered to be involved in APSGN pathogenesis, recent studies have shown that increased anti-SpeB antibody titer and SpeB protein deposition in kidney biopsies are highly correlated with APSGN, which suggests an important role of SpeB in APSGN pathogenesis.[36,37,39,40] In a SpeB-hyperimmunized mouse model, immunoglobulin deposition, complement activation and leukocyte infiltration were found in mouse glomeruli. Furthermore, an anti-SpeB monoclonal antibody was shown to be cross-reactive with kidney endothelial cells and caused kidney injury.[38] These studies suggest that SpeB is an important antigen and anti-SpeB antibody is involved in APSGN pathogenesis.

Conclusion

Although many lines of evidence show that SpeB plays an important role in GAS pathogenesis, some reports indicate that SpeB is not the major virulence determinant in either *in vitro* or *in vivo* models. These conflicting results may be due to the different bacterial strains or different infection models used. In our opinion, the strain-strain diversity among GAS may be the major reason for these discrepancies. CovR/S, the most important two-component regulatory system (controlling ~15% of gene expression in GAS), is considered to be a negative regulator of SpeB.[80,81] However, the *covR* or *covS* spontaneous mutants show the loss of SpeB expression.[79,82] Furthermore, in some strains, mutation of *covR* does not affect SpeB expression.[83,84] These results indicate that the regulatory network that controls SpeB expression in different GAS strains may be different. In addition, the prophages in GAS genomes also increase diversity among different GAS strains.[85,86] Therefore, the diversity among strains may cause different responses when bacteria face the same stimulation. The regulatory network involved in SpeB expression is not completely understood, even today. Understanding how SpeB is regulated by GAS will help us to further elucidate the role of SpeB during GAS infection.

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